Chromatographic Purification of Oligonucleotide-Based Biotherapeutic Products

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Abstract (not used in poster)

The use of oligonucleotides as biotherapeutic agents, whether produced using recombinant or synthetic pathways, continues to grow each year. These include modified and unmodified RNA and DNA based molecules. Traditionally both reversed phase and anion exchange chromatography have been utilized to purify the oligonucleotide, predominantly in an analytical setting. However, as the number of molecules entering into clinical trials continues to increase, pharmaceutical companies are focusing their downstream purification on a single, high-resolution, strong anion exchange chromatography step to generate high purity material. In work previously completed at analytical scale, it was found that better selectivity in anion-exchange chromatography was obtained at a higher pH. The same is true for process scale resins where pH 9.0 was determined to give optimal results for some oligonucleotides. In this study, the dynamic binding capacities for a 24-mer synthetic phosphorothioate oligonucleotide were determined on several anion exchange resins. The capacities ranged from 35 mg/ml to 54 mg/ml. The resins were then challenged with loadings up to 80% of their dynamic capacity and the oligonucleotide isolated using an optimized gradient. The crude (<67% pure) oligonucleotide was purified in a single step using a gradient optimized for this particular molecule. Additionally, and not surprisingly, longer columns (up to 20 cm) gave better resolution between the main peak and the N-1 (and smaller) moieties. The N+1 impurity also was well resolved under these conditions. Fractions with a purity of >85% were pooled and the oligonucleotide purity was >97% and the recovery was 74%. However, a mock pooling study demonstrated that other combinations of high purity and high yield were possible depending on the process demands.

Introduction

Oligonucleotides are short, linear sequences of deoxyribonucleic acid or ribonucleic acid. Because of the unique structure of these molecules and the way they are synthesized, oligonucleotides require special considerations during chromatographic purification.

By comparing the performance of TSKgel® SuperQ-5PW (20), TOYOPEARL ® SuperQ-650S and experimental TOYOPEARL GigaCap ® Q-650S (not yet commercially available) resins at similar loading levels relative to their dynamic binding capacity; the ability of these particular resins to purify oligonucleotides from their impurities was demonstrated.

Experimental Information

The phosphorothioate deoxyoligonucleotide (24-mer) used in this study was supplied unpurified (estimated at 66.5% purity by AEX HPLC) in liquid form from Girindus America Inc. The oligonucleotide is single stranded with a concentration of 981 OD/mL (39.2 mg/mL) by UV at 260 nm and a molecular weight of 7698 amu as a free acid.

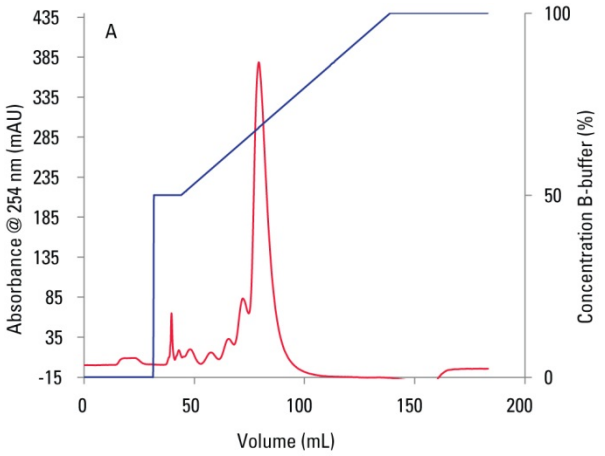
TSKgel SuperQ-5PW (20), TOYOPEARL SuperQ-650S (Tosoh Bioscience LLC) and experimental TOYOPEARL GigaCap Q-650S resin (Tosoh Corporation) resins were packed in 0.66 cm ID × 18.0 ± 0.5 cm columns.

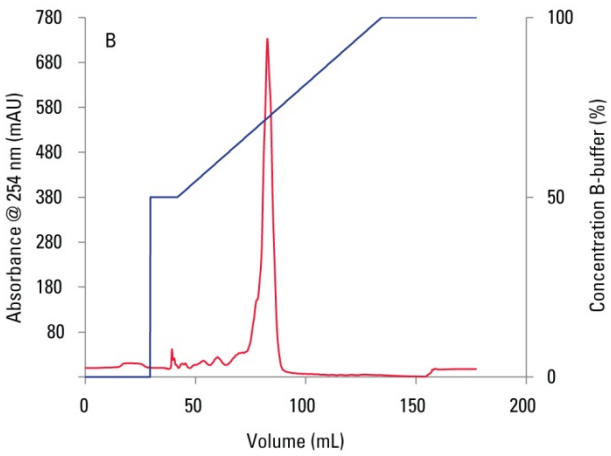
A TSKgel DNA-STAT HPLC column (4.6 mm ID × 10.0 cm) was used for analytical analysis of fractions taken during purification and to determine crude and final oligonucleotide purity.

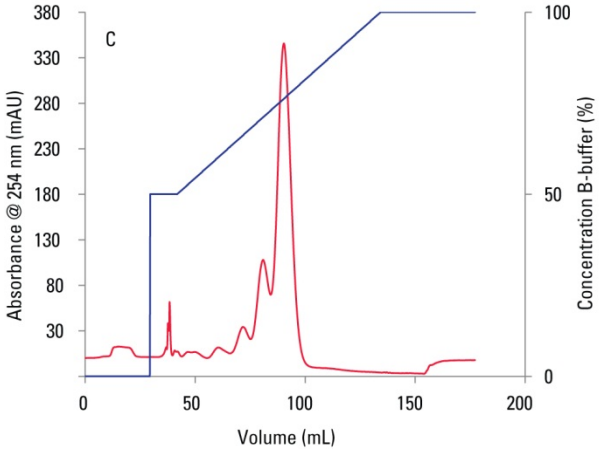
Table 1: Properties of the Anion Exchange Resins Used in this Study

	TSKgel SuperQ-5PW (20)	TOYOPEARL SuperQ-650S	Experimental TOYOPEARL GigaCap Q-650S
Lot#	5QA05RM	65QASB01M	65GQSC503R
Particle size	20 µm	35 µm	30 µm
Pore diameter	1000 Å	1000 Å	1000 Å
Ion exchange capacity	0.14 meq/mL gel	0.26 meq/mL gel	0.17 meq/mL gel
Absorption capacity (BSA)	60 g/L gel	129 g/L gel	189 g/L gel
Recommended Max Pressure	20 bar	3 bar	3 bar

Figure 1: Initial Resin Screening with 1.0 mg Crude Oligonucleotide Load







Resins:	A - TSKgel SuperQ-5PW (20) B – Experimental TOYOPEARL GigaCap Q-650S C – TOYOPEARL SuperQ-650S
Column Size:	0.66 cm ID × 18.0 ± 5 cm
Buffer A:	20 mmol/L NaOH
Buffer B:	20 mmol/L NaOH, 3.0 mol/L NaCl
Gradient:	Step to 50% B (2 CV), 50-100% B (15 CV), 100% B (2 CV)
Flow Rate:	200 cm/hr (1.14 mL/min)
Detection:	UV @ 254 nm
Temperature:	Ambient
Sample:	Crude Oligonucleotide
Sample Load:	1.0 mg
Instrument:	AKTA Explorer

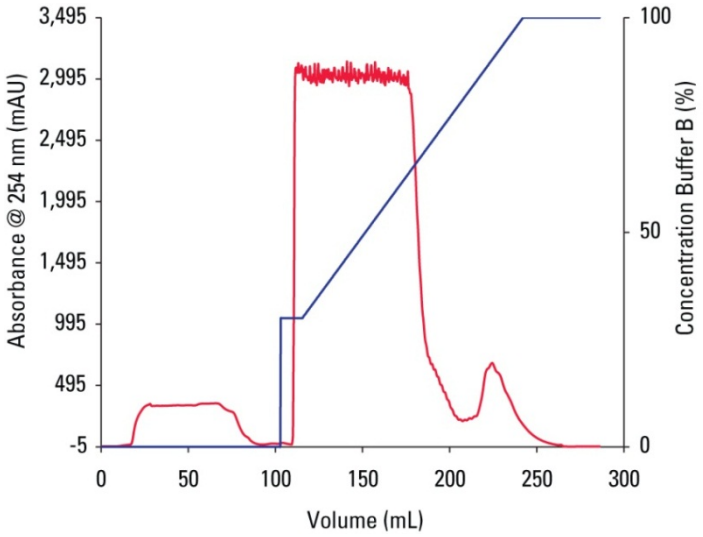
- These chromatograms represent the optimal results obtained for the oligonucleotide used in this study.
- The removal of the N-1 and N+1 impurities indicates the quality of final product purification.

Table 2: Dynamic Binding Capacity of Crude Oligonucleotide

	TSKgel SuperQ-5PW (20)	TOYOPEARL SuperQ-650S	Experimental TOYOPEARL GigaCap Q-650S
DBC @ 10% Breakthrough 200 cm/hr	46.4 mg/mL	53.6 mg/mL	36.8 mg/mL

Resin: Multiple (listed above)
Column Size: 0.30 cm ID × 10.0 cm (0.707 mL)
Buffer A: 20 mmol/L NaOH
Buffer B: 20 mmol/L NaOH, 3.0 mol/L NaCl
Gradient: No Gradient
Flow Rate: 200 cm/hr (0.24 mL/min)
Detection: UV @ 254 nm
Temperature: Ambient
Sample: Crude Oligonucleotide (0.5 mg/mL)
Sample Load: Stop loading at 10% breakthrough
Instrument: AKTA Explorer

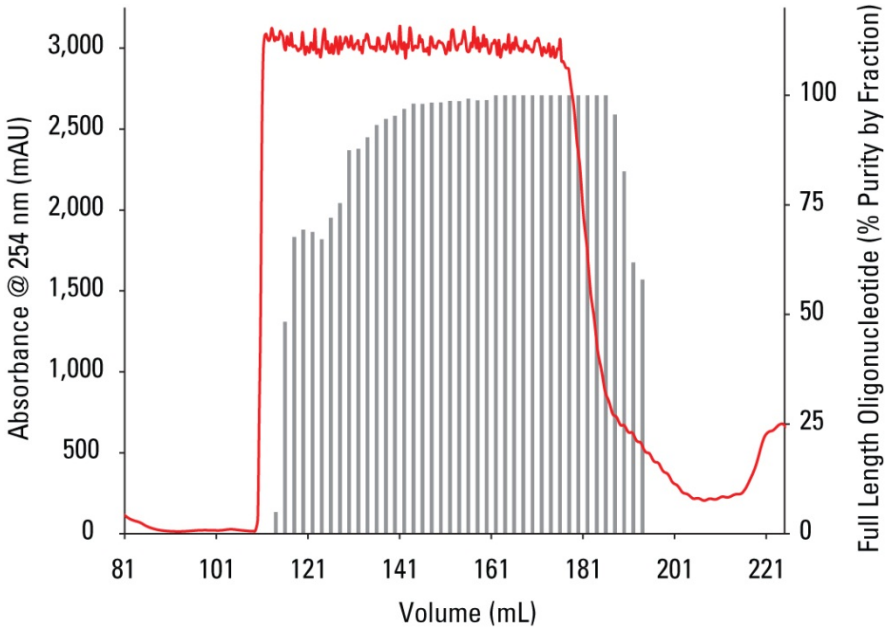
Figure 2: TSKgel SuperQ-5PW (20) Resin – 80% Dynamic Binding Capacity Load



Resin:	TSKgel SuperQ-5PW (20)
Column Size:	0.66 cm ID × 18.5 cm (6.30 mL)
Buffer A:	20 mmol/L NaOH
Buffer B:	20 mmol/L NaOH, 3.0 mol/L NaCl
Gradient:	Step to 30% B (2 CV), 30-100% B (15 CV), 100% B (2 CV)
Flow Rate:	200 cm/hr (1.14 mL/min)
Detection:	UV @ 254 nm
Temperature:	Ambient
Sample:	Crude Oligonucleotide
Sample Load:	80% of Dynamic Binding Capacity (37.1 mg/mL)
Instrument:	AKTA Explorer

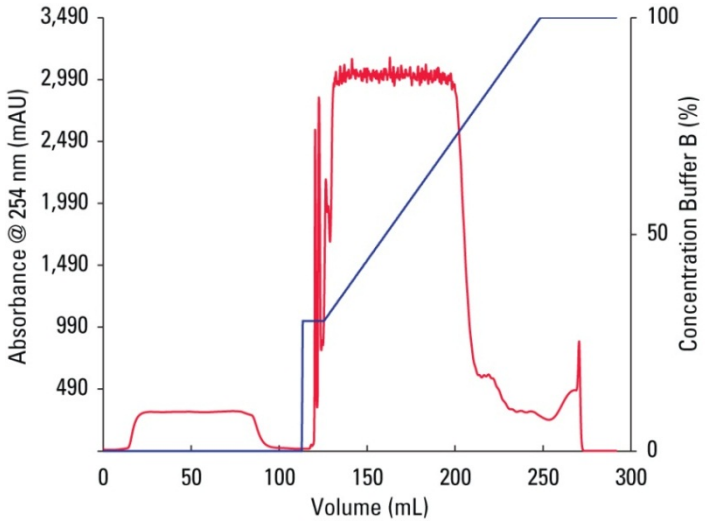
- Crude oligonucleotide was loaded at 80% of the measured dynamic binding capacity
- The N-1 was not resolved in the chromatogram.
- The N+1 was resolved from the main peak.

Figure 3. TSKgel SuperQ-5PW (20) Resin – Fraction Purity Histogram Overlaid



- Individual fractions were analyzed for product purity and plotted along with an expanded view of the chromatogram.

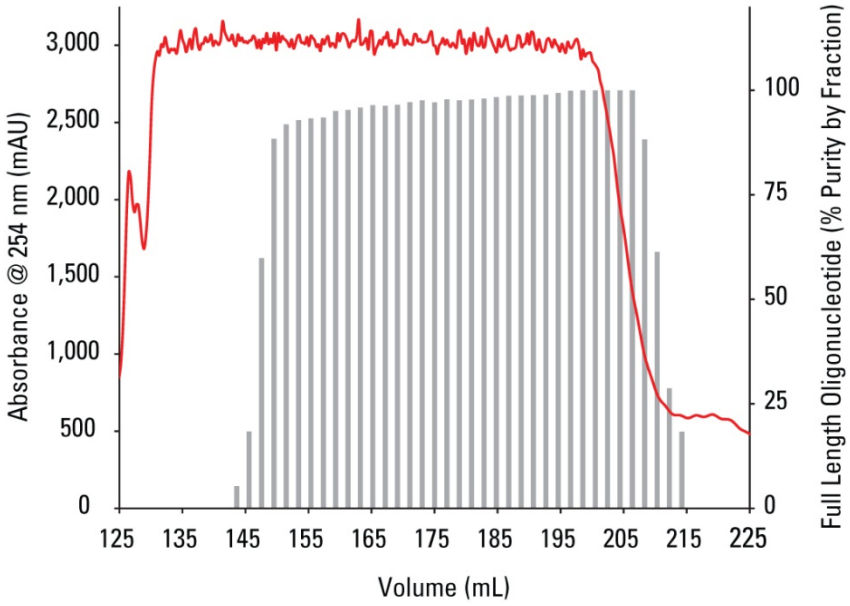
Figure 4. TOYOPEARL SuperQ-650S Resin – 80% Dynamic Binding Capacity Load



Resin:	TOYOPEARL SuperQ-650S
Column Size:	0.66 cm ID × 18.0 cm (6.16 mL)
Buffer A:	20 mmol/L NaOH
Buffer B:	20 mmol/L NaOH, 3.0 mol/L NaCl
Gradient:	Step to 30% B (2 CV), 30-100% B (15 CV), 100% B (2 CV)
Flow Rate:	200 cm/hr (1.14 mL/min)
Detection:	UV @ 254 nm
Temperature:	Ambient
Sample:	Crude Oligonucleotide
Sample Load:	80% of Dynamic Binding Capacity (45.0 mg/mL)
Instrument:	AKTA Explorer

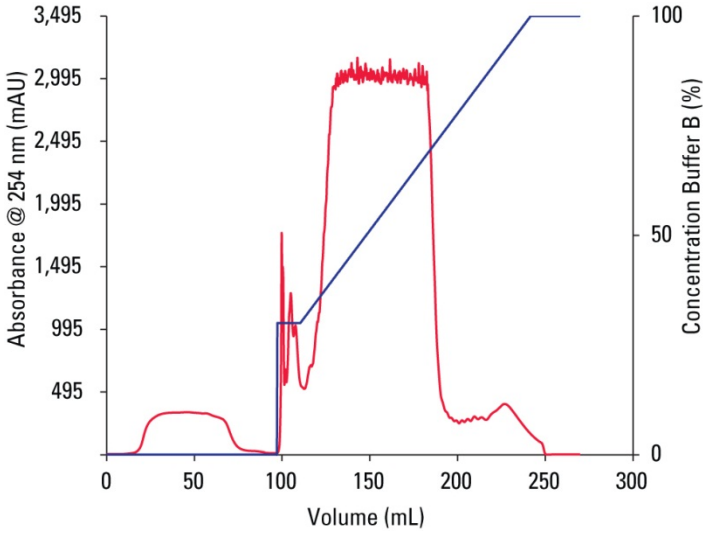
- Crude oligonucleotide was loaded at 80% of the measured dynamic binding capacity.
- The N+1 was not resolved on the chromatogram.
- The N-1 was resolved from the main peak when individual fractions were analyzed by HPLC.

Figure 5. TOYOPEARL SuperQ-650S Resin – Fraction Purity Histogram Overlaid



- Individual fractions were analyzed for product purity and plotted along with an expanded view of the chromatogram.

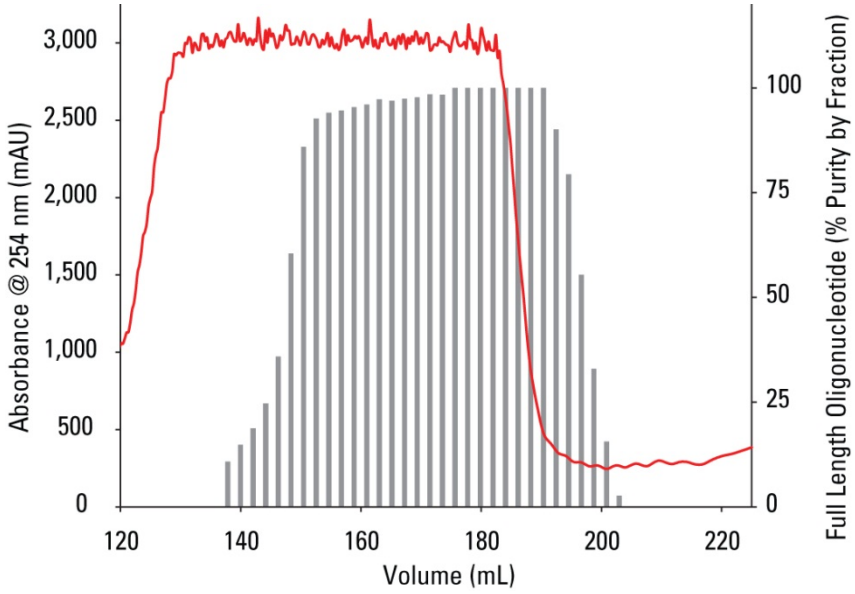
Figure 6. Experimental TOYOPEARL GigaCap Q-650S Resin – 80% Dynamic Binding Capacity Load



Resin:	Experimental TOYOPEARL GigaCap Q-650S
Column Size:	0.66 cm ID × 18.0 cm (6.16 mL)
Buffer A:	20 mmol/L NaOH
Buffer B:	20 mmol/L NaOH, 3.0 mol/L NaCl
Gradient:	Step to 30% B (2 CV), 30-100% B (15 CV), 100% B (2 CV)
Flow Rate:	200 cm/hr (1.14 mL/min)
Detection:	UV @ 254 nm
Temperature:	Ambient
Sample:	Crude Oligonucleotide
Sample Load:	80% of Dynamic Binding Capacity (30.0 mg/mL)
Instrument:	AKTA Explorer

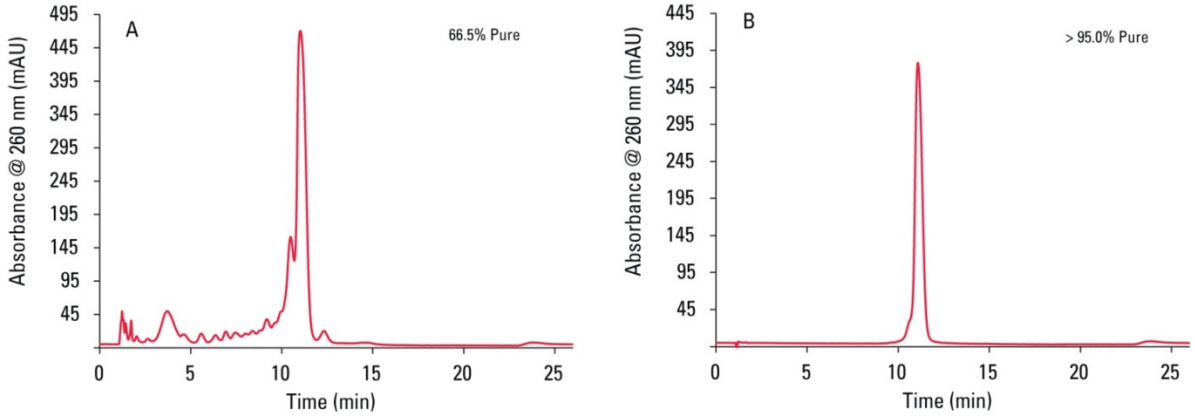
- Crude oligonucleotide was loaded at 80% of the measured dynamic binding capacity
- The N+1 was not resolved on the chromatogram
- The N-1 was resolved from the main peak when individual fractions were analyzed by HPLC

Figure 7. Experimental TOYOPEARL GigaCap Q-650S Resin – Fraction Purity Histogram Overlaid



- Individual fractions were analyzed for product purity and plotted along with an expanded view of the chromatogram
- The pool volume for this resin was calculated to be at least 30% less than the pool volumes of the other two resins tested.

Figure 8. HPLC Analysis of Crude and Purified Oligonucleotide Using a TSKgel DNA-STAT Column



Column:	TSKgel DNA-STAT column, 4.6 mm ID × 10.0 cm
Buffer A:	100 mmol/L Tris, 10% ACN pH 8.0
Buffer B:	100 mmol/L Tris, 2.0 mol/L NaCl, 10% ACN pH 8.0
Gradient:	30-100% B (20 minutes)
Flow Rate:	180 cm/hr (0.5 mL/min)
Detection:	UV @ 260 nm
Temperature:	60° C
Injection Vol.:	10 µL
Sample:	A - crude oligonucleotide B - pure oligonucleotide Pool
Instrument:	Agilent 1100 HPLC

- Purified fractions that were ≥ 85% pure were pooled together and analyzed for overall purity.
- The data indicated that the oligonucleotide was purified to a minimum of >95% for all three resins.

Table 3: Purity and Recovery Results for the Resins at 80% of Dynamic Binding Capacity

Resin	Crude Loaded	Crude Purity (AEX)	Total Recovered (OD 260)	% Full Length Oligo (AEX)	% Yield of FLO (AEX)
TSKgel SuperQ-5PW (20)	37.1 mg/mL	66.5%	118.8 mg	97.3%	74.3%
TOYOPEARL SuperQ-650S	45.0 mg/ mL	66.5%	142.7 mg	95.6%	74.1%
Experimental TOYOPEARL GigaCap Q-650S	30.0 mg/ mL	66.5%	95.3 mg	96.5%	75.0%

Purity and Yield Study

1. The two better resins were loaded to 50, 65, and 80% of the experimentally determined dynamic binding capacity.
2. Fractions were taken across the product peak and analyzed by HPLC.
3. “Mock” pools were assembled for those fractions containing ≥ 80, ≥ 85, ≥ 90, or ≥ 95% pure oligonucleotide.
4. The purity and yield for each “mock” pool were calculated.

Figure 9: Purity Contour Plots

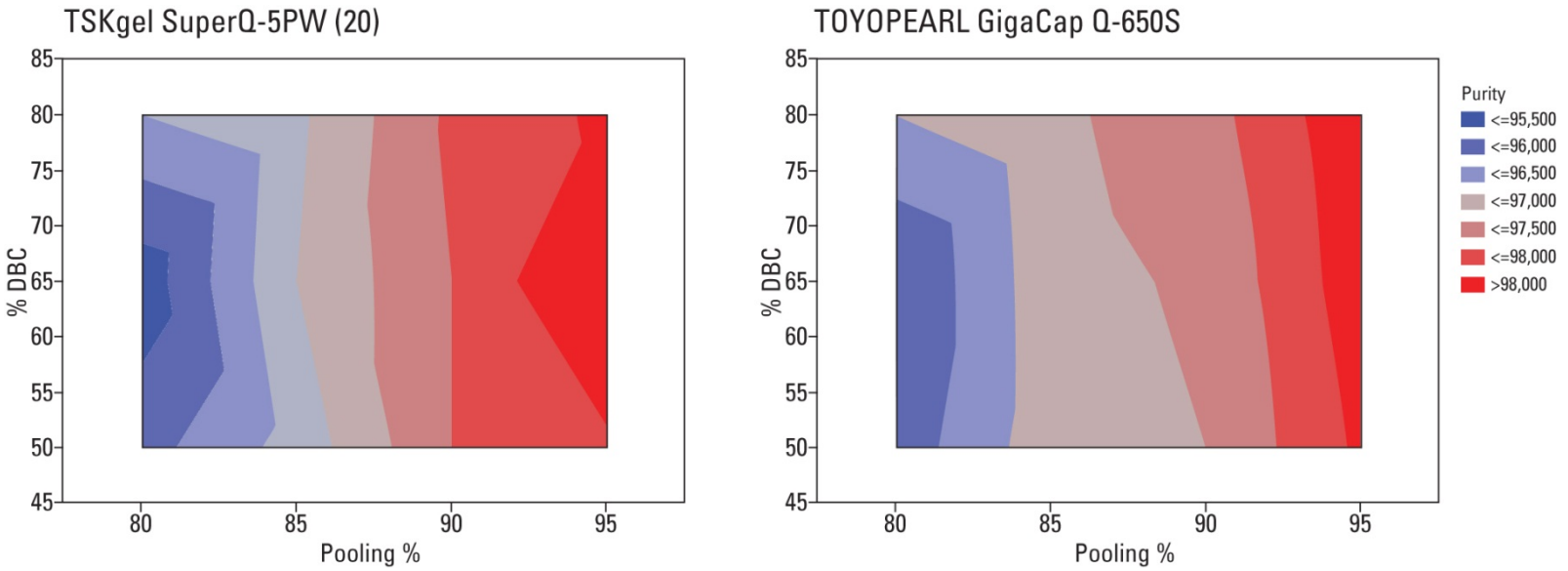
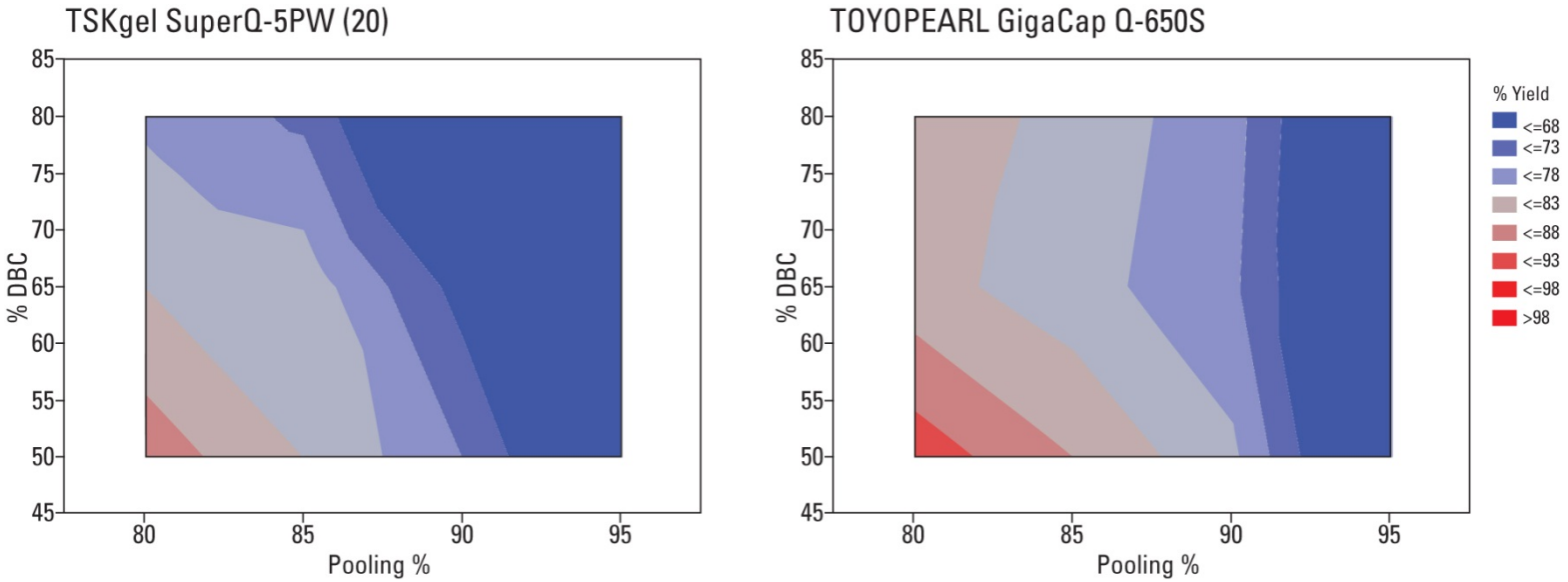


Figure 10: Yield Contour Plots



Conclusions:

1. The TSKgel SuperQ-5PW (46.4 mg/mL), TOYOPEARL SuperQ-650S (53.6 mg/mL) and experimental TOYOPEARL GigaCap Q-650S (36.8 mg/mL) resins exhibited excellent binding capacities for the phosphorothioate oligonucleotide.

2. The experimental TOYOPEARL GigaCap Q-650S pool volume (42 mL) was 30% less than the pool volumes for the TOYOPEARL SuperQ-650S and the TSKgel SuperQ-5PW (20) resins (60 mL).

3. TOYOPEARL SuperQ-650S exhibited the poorest selectivity and produced the lowest purity when loaded to 80% of the dynamic binding capacity and was not included in further studies.

4. Even though the experimental TOYOPEARL GigaCap Q-650S resin has a larger particle size, the purity of the oligonucleotide was essentially equivalent under higher loading conditions compared to the TSKgel SuperQ-5PW (20).

5. Under the highest loading conditions the experimental TOYOPEARL GigaCap Q-650S had higher yields compared to the TSKgel SuperQ-5PW (20) resin.